RESEARCH ARTICLE

Mango-Lime Peel Nanoparticles Improve Diabetic Parameters, Lipid Profiles and Pancreatic Cell Damage in Cigarette Smoke-Exposed Diabetic Rats

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Abstract

BACKGROUND: Diabetes mellitus often exacerbated by cigarette smoke, which disrupts glucose homeostasis and lipid metabolism. Mango (*Mangifera indica* L.) and lime (*Citrus amblycarpa*) peels contain various phytochemical compounds, including those are reported to have metabolic and cytoprotective properties, but their combined nanoparticle formulation in cigarette smoke-exposed diabetes cases remains unexplored. Therefore, this study was conducted to evaluate the synergistic effect of mango-lime peel nanoparticles (NanoMC) on glycemic control, lipid profiles, and pancreatic cell damage in cigarette smoke-exposed diabetic rats.

METHODS: Mango and lime peel ethanol extract was prepared into nanoparticles using emulsion-solvent evaporation method and followed by sonication and homogenization, and subsequently divided into three concentration groups. Rats were diabetic-induced and smoke-exposed, before being treated with/without Simvastatin + Glibenclamide, 20, 40, or 80 mg/kgBW NanoMC. Parameters including blood glucose, insulin, homeostatic model assessment of insulin resistance (HOMA-IR), homeostatic model assessment of beta-cell function (HOMA-β), hemoglobin A1c (HbA1c), and lipid profiles were then measured. To evaluate pancreatic cell damage, the pancreatic tissue was collected, fixed, and then evaluated by using Hematoxylin and Eosin (H&E) staining.

RESULTS: NanoMC administration significantly reduced blood glucose, HbA1c, triglycerides, cholesterol, and low-density lipoprotein (LDL) levels ($p<0.05$). NanoMC treatment also increased insulin levels, HOMA-β, and high-density lipoprotein (HDL). After the treatment with NanoMC, particularly with 80 mg/kgBW concentration, fewer degenerative and necrotic cells were found compared to the other groups, showing its ability to attenuate pancreatic cell damage.

CONCLUSION: The combination of NanoMC could improve glycemic status, modulate lipid profiles, and improved pancreatic cell damage in diabetic rats exposed to cigarette smoke.

KEYWORDS: *Mangifera indica*, *Citrus amblycarpa*, nanoparticles, diabetes mellitus, cigarette smoke, lipid profiles, pancreatic histopathology

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Introduction

Diabetes mellitus is a chronic metabolic disease that affects around 422 million people and is directly responsible for 1.5 million fatalities annually. It is the seventh greatest cause of mortality globally. This illness affects 6.9% of Indonesians over the age of 15.(1) Type 2 diabetes mellitus (T2DM) is distinguished by decreased insulin production, insulin resistance, chronic hyperglycemia, and frequent

abnormalities in lipid metabolism.(2) Pharmacological medication and lifestyle changes that improve glucose control and lower cardiovascular risk are well-established therapeutic treatments for T2DM.(3) Diabetes are multifactoral disease, but it was reported that oxidative stress and inflammation have significant roles in diabetes progression, that might further lead to β-cell malfunction, insulin resistance, and dyslipidemia.(2-4)

Cigarette smoking is also a significant environmental factor that can exacerbate metabolic dysfunction in diabetes progression.(5) Nicotine and other cigarette smoke ingredients may influence glucose homeostasis by acting on nicotinic acetylcholine receptors and metabolic organs such as pancreatic islets, liver, skeletal muscle, adipose tissue, and inflammatory cells.(5,6) Associations between smoking, oxidative stress, inflammation, insulin resistance, and cardiovascular risk were also reported in several experimental and clinical studies.(5-7)

Despite significant advances in diabetes management and smoking exposure therapies, the current interventions primarily effective in regulating blood glucose levels, but generally do not improve the complex metabolic dysfunctions that drive disease progression. Therefore, it is highlighting the need for therapeutic strategies that address the fundamental mechanisms underlying both conditions. Recently herbal compounds are found to be highly beneficial for the management of diabetes and have antioxidants properties, as well. (8) Prior phytochemical analysis of mango (*Mangifera indica*) and lime (*Citrus amblycarpa*) peel ethanol extracts demonstrated the presence of alkaloids, polyphenols, flavonoids, anthraquinones, tannins, and terpenoids, and showed DPPH radical-scavenging activity that may have potential metabolic and cytoprotective properties.(9-11) These previous findings support a rationale for selecting mango-lime peel extracts as phytochemical-rich materials.

Typical plant extracts may have disadvantages, such as low water solubility, variable chemical stability, limited absorption, and decreased bioavailability of several polyphenolic chemicals.(12) Nanoparticle-based delivery strategies have been proposed to increase polyphenol dispersion, stability, absorption, and biological availability. (12,13) As a result, the nanoparticle formulation used in this study was chosen to boost the transport potential of bioactive chemicals found in mango-lime peel. Therefore, this study was conducted to determine the effects of mango-lime peel nanoparticles (NanoMC) on glycemic indices, lipid profiles, and pancreatic cell damage in diabetic rats exposed to cigarette smoke.

Methods

Preparation of Mango-Lime Peel Nanoparticles

The mango (*Mangifera indica* L.) and lime (*Citrus amblycarpa*) fruits used in this study were grown in Bandung and botanically authenticated at the Herbarium of the School of Life Sciences and Technology (SITH), Institut Teknologi Bandung (ITB), Bandung, Indonesia. Ethanol extracts of mango and lime peels were combined at a 1:1 ratio. The extract mixture was dissolved in dimethyl sulfoxide (DMSO), alcohol, and ethyl acetate, and then incorporated into poly lactic-co-glycolic acid (PLGA) polymer solution to form the organic phase. Aqueous polyvinyl alcohol (PVA) and chitosan solutions were used as the water phase. The organic phase was added dropwise into the aqueous phase under magnetic stirring, followed by probe sonication in an ice bath, homogenization using Ultra-Turrax, solvent evaporation, and addition of sodium carboxymethyl cellulose (Na-CMC) solution to obtain a homogeneous nanoparticle suspension.

Cigarette Smoke-Exposure

In this study, the high-fat diet and whole-body cigarette smoke exposure were given concurrently for 28 days before diabetes induction. During this period, rats in the negative control, positive control, and NanoMC-treated groups received a high-fat diet and were exposed to smoke from six cigarettes per day in an exposure box. Lipid-profile assessment was then performed to confirm hypercholesterolemia. Afterward, diabetes was induced using streptozotocin to establish the cigarette smoke-exposed diabetic rat model. The passive smoking rat model for this research was equipped with a cigarette holder and electric fan to optimize cigarette combustion, thus creating individual chamber passive smoking model.

In this study, the design of the cage were modified by placing the cigarette holder at the bottom of the cigarette compartment with a cage size of 100 cm x 60cm x 60cm and made of acrylic (Supplementary 1). This exposure level was chosen as a subchronic procedure that has been used in studies on diabetic rats and experimental metabolism. Exposure to roughly six cigarettes over the course of four weeks during a one-hour session has been linked to increased oxidative stress and inflammatory responses in diabetic rats. Additionally, exposure of six cigarettes with 2 mg nicotine per stick had worsened hyperglycemia and other metabolic parameters in rats fed a high-fat diet.(14,15)

Local unfiltered cigarettes which contained 2.0 mg of nicotine and 44 mg of tar (Dji Sam Soe, PT Hanjaya Mandala (HM) Sampoerna, Surabaya, Indonesia) was used. Subjects were exposed to cigarette smoke from six cigarettes daily, resulting in an estimated daily exposure of 12 mg of nicotine and 264 mg of tar for 28 days. The cigarette smoke-exposure was interpreted on the basis of the administered protocol and relevant prior evidence rather than on biomarker-confirmed internal exposure.

Animal Grouping and Treatment

Before separated into groups, all rats were acclimatized for 7 days. Thirty-six male Sprague Dawley rats were allocated into six groups: one healthy normal-control group (NG) and five diabetes-induced cigarette smoke-exposed groups. Other than the rats in the NC group, the rats received HFD during the first 28 days (day-0 to day-28), concurrently with daily cigarette smoke exposure as described above. After the lipid profile measurement was performed to confirm hypercholesterolemia, the non-normal control groups then received diabetes-induction by the administration of nicotinamide (NA) (Sigma-Aldrich, St. Louis, MO, USA) for five days and single dose intraperitoneal injection of 60 mg/kg BW streptozotocin (STZ) (Sigma-Aldrich). The day after, the diabetic induction was confirmed by examining the blood glucose level. After the confirmation of diabetes, the five non-normal control groups then received either no treatment (NCG), 20 mg/kg BW simvastatin and 5 mg/kg BW glibenclamide as positive control (PCG), NanoMC at 20 mg/kg BW (TG1), 40 mg/kg BW (TG2), or 80 mg/kg BW (TG 3) for 28 days. The dose rationale for the PCG was based on prior rat studies using glibenclamide as an antidiabetic comparator (16), and simvastatin/statins as lipid-lowering comparators in diabetic or dyslipidemic rat models (17). Meanwhile, the NC group received normal diet and fresh air throughout the study. On the last day of the study, the rats were measured for several blood glucose parameters (glucose, insulin, homeostatic model assessment of insulin resistance (HOMA-IR), homeostatic model assessment of beta-cell function (HOMA-β), hemoglobin A1c (HbA1c)) and lipid profile parameters (trygliseride, total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (Figure 1). All procedures were documented in Supplementary 2.

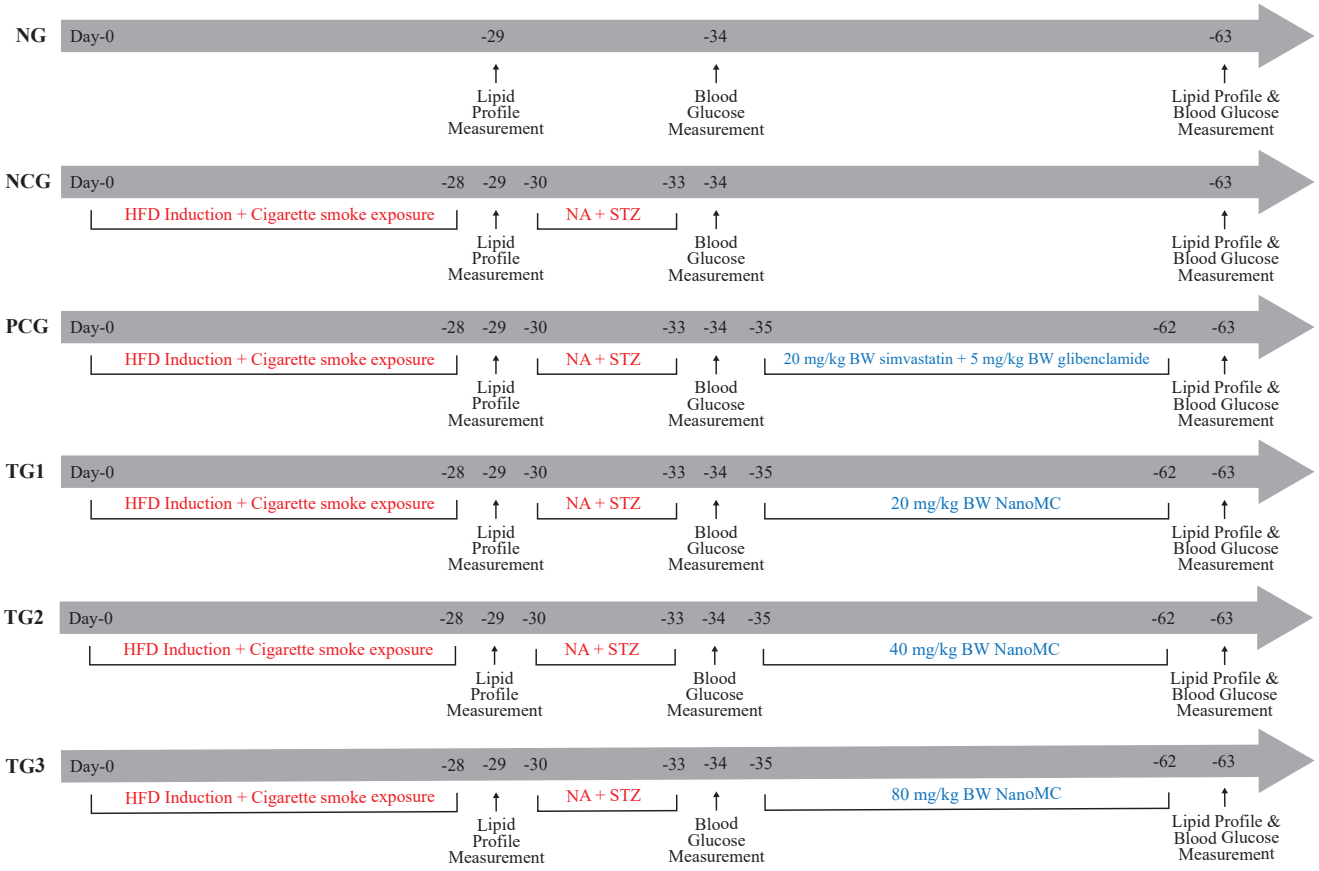

Figure 1. Schematic diagrams of study design and timelines. NC: normal control; NGC: negative control group; PGC: positive control group; TG1: treatment group 1; TG2: treatment group 2; TG3: treatment group 3.

Blood Sample Collection

For the pre-intervention measurement, to ensure the survival of the experimental animal during the blood sampling, the volume collected did not exceed 10% of the estimated total blood volume within the recommended recovery interval.(18) Meanwhile, a larger blood collection of approximately 3 mL was performed only as terminal sampling under anesthesia at the end of the experiment and was not repeated as survival bleeding. Tail blood collection was performed after warming for vasodilation, followed by skin disinfection, blood collection using a 3 mL syringe, hemostasis, and povidone iodine application. For the pre-intervention measurement, the rats were returned to the cage after recovery monitoring.

Glycemic Profile Measurement

The measurement of fasting blood glucose, HbA1c, insulin levels, HOMA-IR, HOMA-β was carried out twice, which were after the diabetic induction (pre-value) and at the end of the study (post-value). Blood glucose measurements were carried out using the enzymatic method. Meanwhile, the insulin and HbA1C were measured using Enzyme-Linked Immunosorbent Assay (ELISA) methods with commercially available ELISA kits as follows: Rat INS (Insulin) ELISA Kit (Cat. No. ER1113; FineTest, Boulder, CO, USA) with detection range of 78.125-5,000 pg/mL and sensitivity of 46.875 pg/mL, and Rat HbA1C (Glucosylated Hemoglobin/ Hemoglobin A1c) ELISA Kit (Cat No. ER1030; FineTest) with detection range of 1.563-100 ng/mL and sensitivity of 0.938 ng/mL, respectively. Insulin resistance was then calculated using the HOMA-IR and HOMA-β index with already established formula.(19)

Lipid Profile Measurements

Cholesterol, triglyceride, and HDL were assessed using precipitation reagent for in vitro determination of cholesterol serum, according to the cholesterol oxidase–peroxsidase aminoantypirin) (CHOD PAP) method on photometric systems (colour intensity photometrically 480 and 520 nm). Quinoneimine was used as colorimetric indicator, which was generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinder's reaction). Ten µL sample and blank were incubated for 20 minutes at 20 – 25°C or for 10 minutes at 37°C, and the absorbance was read within 60 min against reagent blank.

Meanwhile, the concentration of LDL cholesterol was calculated as the difference between total cholesterol and cholesterol in the supernatant. A 100 µL sample and 1000 µL precipitating reagent were mixed and incubated for 15 minutes at room temperature, then centrifuged for 20 minutes at 2500 g. Within one hour after centrifugation, 100 µL of the clear supernatant was transferred to the reaction solution for the determination of cholesterol. Then the supernatant and standard were incubated for 10 minutes at room temperature or 5 minutes at 37°C, and the absorbance of the sample was read within 45 minutes against the reagent blank.

Assessment of Pancreatic Cell Damage

Rats were anesthetized with ketamine prior to tissue extraction. Pancreas tissues were harvested, fixed in formalin, and processed for histological analysis. Pancreatic sections were stained with Hematoxylin and Eosin (HE) for microscopic examination at 100x and 400x magnifications by Olympus CX-23 (Olympus, Tokyo, Japan).

Qualitative analysis of the pancreas tissue was performed by assessing the degree of islet of Langerhans cell damage using a semiquantitative scoring system adapted from prior research.(14) The scoring criteria were as follows: Score 0: No visible changes were observed in the border of the islets of Langerhans; no necrotic cells were present; the number of cells was preserved; and the cellular morphology remained normal. Score 1: The islet border was clearly visible; the number of cells began to decrease; no necrotic cells were observed, but cellular degeneration was present; and the cellular morphology was still relatively well preserved. Score 2: The islet border began to appear indistinct; the number of cells was further reduced; cellular degeneration was present; and some cells showed abnormal morphology. Score 3: The islet border was not clearly visible; the number of cells was markedly reduced; necrotic cells were present; and many cells showed abnormal morphology. Score 4: The islet border was very indistinct; the number of cells was significantly reduced; almost all cells showed necrotic changes; and marked cellular morphological alterations were observed.

Statistical Analysis

Data are presented as mean±standard deviation (SD). Statistical analysis was performed by Normality test conducted using Shapiro-Wilk Goodness of Fit Test and the test results with $\alpha = 5\%$, at 95% confidence level, were normally distributed ($p>0.05$). Diabetic and lipid profile from blood sample were analyzed by Two-Way Repeated Measures ANOVA, followed by Tukey's post-hoc test using GraphPad Prism 9.5 (GraphPad Software, San Diego, CA, USA). Pancreatic histopathology was performed using the Kruskal–Wallis test followed by Dunn's post-hoc test

with Bonferroni correction. Differences were considered statistically significant at $p<0.05$.

Results

Baseline Body Weight of Experimental Rats

Body weight was measured during the adaptation/baseline phase prior to treatment allocation (Table 1). The normality test using the Shapiro-Wilk test yields a normal distribution ($p>0.05$). In terms of body weight, the ANOVA test revealed a significant difference. Body weight was included to describe the baseline physical condition of the rats before intervention and to support comparability among experimental groups. Although the overall ANOVA showed a significant difference among groups, the treatment groups had similar body weights, and this baseline measurement should not be interpreted as a treatment effect because it was recorded before administration of NanoMC or positive-control drugs.

NanoMC Treatment Lowered Glycemic Parameters

The glycemic parameters, including fasting blood glucose, insulin, HOMA-IR, HOMA-β, and HbA1c were measured twice: after diabetes induction and before treatment administration for the pre-treatment value; and at the end of the treatment period as the post-treatment value (Table 2). Each parameter showed significant differences between groups ($p<0.001$). Compared with the NCG, the PCG and NanoMC-treated groups showed improvement in glycemic parameters after the treatment, characterized by lower fasting blood glucose, HOMA-IR, and HbA1c and higher insulin and HOMA-beta values after treatment.

NanoMC Treatment Improved Lipid Profiles

Lipid profiles, including triglycerides, total cholesterol, HDL, and LDL, were also measured twice: after diabetes induction and before treatment administration for the pre-treatment value; and at the end of the treatment period, as the post-treatment value (Table 3). In the PCG, TG1, TG2, and TG3 groups, triglyceride, total cholesterol, and LDL levels decreased after treatment, while HDL levels increased. There were significant differences between all groups for all lipid profile parameters changes ($p<0.001$). The strongest overall improvement among the NanoMC groups was generally observed in TG3, supporting a dose-related metabolic response. These findings indicate that NanoMC treatment was associated with improved lipid regulation in cigarette smoke-exposed diabetic rats.

NanoMC Treatment Attenuated Damaged Cells in Pancreas Tissue

Microscopic view of pancreatic tissue (Figure 2) showing differences among the experimental groups. In general, the most severe pancreatic cell damage was observed in diabetic rats exposed to cigarette smoke (NCG). PCG, TG1, TG2, and TG3 showed different degrees of structural improvement, but with distinct fewer degenerative and necrotic cells compared to NCG.

There was a significant difference between the groups according to the semiquantitative scoring of islet of Langerhans cell damage ($p<0.001$). Severe islet injury was indicated by the NCG's highest damage score (3.83±0.41). The NC group, on the other hand, had no discernible islet damage (0.00±0.00). The damage score for the PCG was substantially lower than that of the NCG group (0.50±0.55, $p<0.01$). TG3 had the lowest damage score among the NanoMC-treated groups (0.50±0.55), which was substantially lower than the NCG's ($p<0.01$) (Table 4). Although TG1 and TG2 had lower damage scores than the negative control group, post-hoc analysis revealed that the differences were not statistically significant.

Discussion

Diabetes is one of the most complex disorders caused by β-cell dysfunction, leading to poor insulin release

Table 1. Rats' body weight during the adaptation period (baseline).

Body Weight (gram)	Groups						*p* -value
	NG	NCG	PCG	TG 1	TG 2	TG 3	
Mean±SD	212.78±3.4	238.61±2.6	246.88±2.8	246.35±2.9	245.48±2.4	246.71±3.0	<0.001*
Median	212.10	238.75	247.4	245.7	244.8	246.75	
(Min-Max)	(208.3-217.7)	(235-242)	(242.4-250.2)	(242.7-250.9)	(243.-248.6)	(243.4-250.6)	

The significant difference between all group's mean vaue was calculated using one-way ANOVA, *$p<0.05$ is considered significant. The significant difference shows that before the treatment, all the induced groups showed higher body weight compared to the NG. Meanwhile, the NCG, PCG, and treatment groups (TG1, TG1, and TG3) show similar body weight before the treatment.

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Table 2. Blood glucose profile levels on experimental rats.

Group	Fasting Blood Glucose (mg/dL)[a]			Insulin (pg/dL)[b]			HOMA-IR[b]			HOMA-β[a]			HbA1c (ng/dL)[b]		
	Pre-T	Post-T	Δ	Pre-T	Post-T	Δ	Pre-T	Post-T	Δ	Pre-T	Post-T	Δ	Pre-T	Post-T	Δ
NG	68.24±1.40	69.39±1.40	1.14±0.22	545.68 (536.21–552.00)	542.78 (532.64–546.67)	-2.87 (-5.33–(-0.55))	2.75 (2.68–2.81)	2.79 (2.71–2.83)	0.03 (0.02–0.04)	69.22±17.08	55.92±12.08	-13.30±5.95	28.91 (28.30–29.29)	29.46 (28.85–30.07)	0.55 (0.44–0.78)
NCG	269.03±2.65	270.16±2.71	1.12±0.67	468.32 (463.58–474.63)	466.37 (457.80–473.39)	-1.98 (-8.94–(-1.11))	9.34 (9.22–9.47)	9.30 (9.19–9.51)	0.005 (-0.17–(-0.04))	1.68±0.02	1.67±0.02	-0.01±0.007	72.01 (69.03–73.33)	73.11 (70.35–74.55)	1.21 (0.99–1.32)
PCG	268.85±3.42	111.68±3.05	-157.17±6.1	462.79 (457.26–469.89)	510.03 (501.46–513.93)	47.20 (39.36–50.43)	9.19 (9.08–9.49)	4.23 (4.08–4.36)	-4.90 (-5.21–(-4.85))	1.68±0.026	7.10±0.45	5.42±0.43	72.78 (71.90–74.44)	37.90 (36.91–38.90)	-35.15 (-36.76–(-33.56))
TG1	267.21±4.18	124.01±2.71	-144.2±6.11	465.95 (455.68–477.79)	501.46 (495.22–507.69)	33.96 (27.17–52.01)	9.22 (8.97–9.56)	4.55 (4.46–4.75)	-4.66 (-5.10–(-4.31))	1.69±0.038	5.75±0.26	4.06±0.27	72.62 (71.02–74.00)	46.68 (45.52–47.73)	-25.60 (-28.48–(-23.39))
TG2	268.55±3.17	100.41±2.20	-168.13±3.3	451.74 (432.00–465.16)	513.15 (509.25–521.73)	62.23 (48.77–89.73)	8.95 (8.64–9.31)	3.82 (3.70–3.95)	-5.18 (-5.41–(-4.82))	1.68±0.02	9.23±0.54	7.54±0.53	73.06 (71.79–74.33)	36.80 (36.14–37.68)	-36.12 (-37.64–(-35.43))
TG3	270.19±1.75	90.92±2.90	-179.27±4.1	456.47 (444.63–462.00)	530.30 (526.40–537.32)	73.86 (70.66–84.89)	9.16 (8.93–9.19)	3.59 (3.40–3.72)	-5.52 (-5.67–(-5.43))	1.67±0.019	12.43±1.30	10.76±1.31	73.44 (72.89–74.33)	31.89 (31.50–32.16)	-41.39 (-42.83–(-41.17))
p-value			<0.001*			<0.001*			<0.001*			<0.001*			<0.001*

Pre-treatment (Pre-T) values were measured after diabetes confirmation and before treatment administration; post-treatment (Post-T) values were measured at the end of the 28-day intervention period. [a]Data are presented as mean±SD; [b]Data are presented as median (minimum-maximum). Δ values were calculated as post-treatment minus pre-treatment values. Negative Δ values indicate a reduction after treatment, whereas positive Δ values indicate an increase after treatment. The *p*-values refer to between-group comparisons of Δ values and were calculated using two-way repeated-measures ANOVA followed by Tukey's post-hoc test for parametric variables, or the Kruskal-Wallis test followed by Dunn's post-hoc test with Bonferroni correction for non-parametric variables. *p<0.05 is considered significant.

Table 3. Blood lipid profile levels pre and post-treatment on experimental rats.

Group	Triglyceride (mg/dL)[a]			Cholesterol (mg/dL)[a]			HDL (mg/dL)[b]			LDL (mg/dL)**		
	Pre-T	Post-T	Δ	Pre-T	Post-T	Δ	Pre-T	Post-T	Δ	Pre-T	Post-T	Δ
NG	71.2±2.25	72.36±2.36	1.1±0.53	87.98±2.58	89.21 ±2.53	1.23±0.46	82.01 (77.70–84.89)	83.27 (79.55–85.50)	1.26 (0.61–1.85)	26.49 (24.63–28.36)	27.92 (25.44–29.68)	1.34 (0.78-1.44)
NCG	137.33±4.55	138.79±4.45	1.45±4.43	213.38±4.65	215.80±5.08	2.42±1.36	25.18 (21.58–27.34)	24.16 (20.82–26.77)	-0.70 (-1.34–(-0.57))	78.36 (75.37–81.34)	79.15 (76.33–82.69)	0.80 (0.15-1.35)
PCG	133.33±2.30	88.49±1.53	-44.84±2.67	215.73±2.71	100.73±1.91	-115.±23.15	24.46 (20.86–26.62)	64.31 (62.45–66.91)	40.88 (37.32–41.73)	78.73 (76.87–81.34)	36.40 (32.51–38.16)	-43.45 (-46.71-(-38.32)
TG1	131.92±2.30	104.62±1.68	-27.29±3.09	215.61±3.18	133.57±3.93	-82.03±6.19	22.66 (20.14–25.18)	51.67 (49.81–56.51)	29.37 (25.38–34.21)	78.36 (76.12–82.84)	46.29 (44.52–50.18)	-30.68(-38.32-(-28.18)
TG2	132.12±3.63	85.76±2.87	-46.29±5.46	214.74±2.55	99.75±3.50	-114.99±3.9	23.38 (22.30–24.46)	65.80 (62.45–67.66)	42.42 (37.99–44.64)	77.24 (76.12–79.85)	34.28 (32.51–38.87)	-43.70(-46.63-(-37.25)
TG3	132.98±1.75	81.73±1.04	-51.25±2.51	217.59±2.47	94.85±2.13	-112.74±2.5	24.82 (22.30–26.62)	73.61 (70.63–76.58)	48.05 (46.24–52.12)	80.97 (79.10–82.84)	31.45 (26.15–34.63)	-48.02(-55.49-(-46.71)
p-value			<0.001*			<0.001*			<0.001*			<0.001*

Pre-treatment (Pre-T) values were measured after diabetes confirmation and before treatment administration; post-treatment (Post-T) values were measured at the end of the 28-day intervention period. [a]Data are presented as mean±SD; [b]Data are presented as median (minimum-maximum). Δ values were calculated as post-treatment minus pre-treatment values. Negative Δ values indicate a reduction after treatment, whereas positive Δ values indicate an increase after treatment. The *p*-values refer to between-group comparisons of Δ values and were calculated using two-way repeated-measures ANOVA followed by Tukey's post-hoc test for parametric variables, or the Kruskal-Wallis test followed by Dunn's post-hoc test with Bonferroni correction for non-parametric variables. *p<0.05 is considered significant.

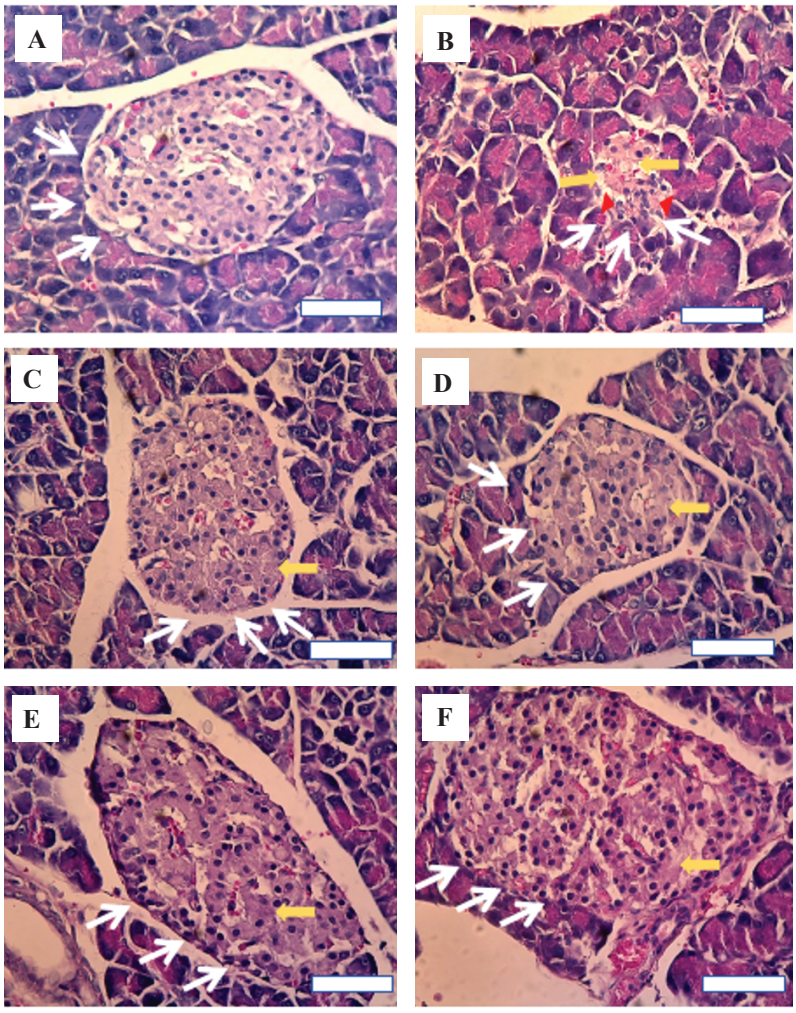

Figure 2. Pancreatic cell damage in experimental rats assessed by using HE stain, focusing on the architecture of islet of Langerhans. A: NG; B: NCG; C: PCG; D: TG1; E: TG2; F: TG3. The white arrow indicates the border of the islet cells; The orange arrow indicates the presence of degenerated cells; The red arrowhead indicates necrotic cells. White bar: 100 µm.

from these cells or poor function of the released insulin. By inflammation, lipolysis, insulin resistance, and cardiovascular risk, cigarette smoke may exacerbate this metabolic disruption.(15,20) Treatment for diabetics exposed to cigarette smoke is currently being developed with herbal medicine. A variety of chemical compounds called secondary metabolites are present in plants. These phytochemicals are produced as metabolic by-products and play an important role in plant protection.(21) In the current animal study, it was shown that in diabetic rats exposed to cigarette smoke, mango-lime peel nanoparticles enhanced pancreatic islet shape, lipid profiles, and glycemic indices. The group received 80 mg/kg BW NanoMC had the highest overall response, with higher insulin, HOMA-β, and HDL levels and significant decreases in fasting blood glucose, HbA1c, triglyceride, total cholesterol, and LDL levels. These results imply that NanoMC may function as a modulator of diabetes-related dyslipidemia and pancreatic structural damage in addition to acting as an antihyperglycemic therapeutic.

The observed improvement in glycemic parameters may be partly related to phytochemical-mediated modulation of glucose metabolism. The biological effects of mango-lime peel nanoparticles observed in the present study may be related to the phytochemical constituents of both peel extracts and their antioxidant properties. Previous phytochemical analysis of mango and lime peel ethanol extracts demonstrated the presence of alkaloids, polyphenols, flavonoids, anthraquinones, tannins, and terpenoids, and showed antioxidant activity using the DPPH method.(13) Dietary intake of polyphenols, especially phenolics, flavonoids, tannins, and coumarins, is associated with a decreased prevalence of T2DM. Natural fruits with high polyphenol content can regulate carbohydrate metabolism through various methods, including maintaining and improving beta-cell function and increasing insulin release activity and cellular glucose uptake, modulation of glucose release from the liver, modulation of intracellular signaling pathways, activation of insulin receptors, and gene expression.(22-24)

Table 4. Langerhans cell damage scoring.

Group	Langerhans Cell Damage Scoring						Mean±SD
	1	2	3	4	5	6	
NG	0	0	0	0	0	0	0.00±0.00***
NCG	4	4	4	4	4	3	3.83±0.41
PCG	0	1	1	0	1	0	0.50±0.55**
TG1	3	2	2	2	2	3	2.33±0.52
TG2	2	2	2	1	2	1	1.67±0.52
TG3	1	0	0	1	1	0	0.50±0.55**

Data are presented as mean±SD. Statistical analysis was performed using the Kruskal–Wallis test followed by Dunn's post-hoc test with Bonferroni correction. The Kruskal–Wallis test showed a significant difference among groups, with **p<0.01 and ***p<0.001 compared with the NGC.

The antihyperglycemic effects of mango-lime peel nanoparticles are consistent with findings that plant-derived bioactive chemicals can influence glucose homeostasis via multiple mechanisms. *Muntingia calabura* L. leaf water extract had anti-diabetic properties in both insulin-deficient and insulin-resistant animal models. In the insulin-deficient animal, the extract reduced fasting blood glucose in a dose-dependent manner, whereas in the insulin-resistant model, it increased the constant of insulin tolerance, indicating increased insulin sensitivity.(26) These findings lend support to the hypothesis that phytochemical-rich extracts may improve diabetes parameters not only by lowering blood glucose but also by modulating insulin activity. *Polysaccharide peptide* from *Ganoderma lucidum* is significantly affected lipid component in diabetic-rat model (27), which is similar to metabolite identified in mango peel.

Furthermore, lime-derived bioactive chemicals have been shown to treat lipid abnormalities; for example, lime essential oil lowered serum total cholesterol, triglycerides, and LDL cholesterol in rats fed a high-fat diet that caused hyperlipidemia.(28) Recent study's improvement in lipid profiles is also in line with earlier experimental findings. *G. lucidum* polysaccharide peptide decreased the number of aortic foam cells and enhanced lipid components in a T2DM rat model. Reductions in triglyceride, total cholesterol, and LDL levels in the current study may indicate better lipid management as a result of enhanced insulin action, decreased oxidative stress, and phytochemical-mediated modulation of lipid metabolism.(27)

HDL measurements revealed that practically all treatment groups (except the normal group) saw an increase in HDL, followed by a drop in LDL. The rise in HDL reported in the PCG group should not be assumed as a direct HDL-targeting impact of glibenclamide. Simvastatin may cause a slight elevation in HDL-C in some cases, whereas glibenclamide is predominantly an antihyperglycemic drug and is not generally thought to have a direct HDL-raising effect. In the current study, HDL improvement might have occurred indirectly as an outcome of better overall metabolic regulation, such as decreased hyperglycemia, a lower triglyceride-rich lipoprotein burden, and enhanced insulin action.

Besides mango, lime are also known to improved blood total cholesterol, triglycerides, low-density lipoprotein cholesterol, alanine aminotransferase, and aspartate transaminase levels in hyperlipidemic rats and simultaneously, lime essential oil enhanced the rats' health in terms of obesity, atherogenic index, and fatty livers. Lime contains vitamin C, which lowers blood leptin levels, boosts L-carnitine production, improves lipid profile, FBG, and lowers inflammatory biomarkers in obese persons. (28,29)

In the current study, the pancreatic islets of Langerhans in untreated diabetic smoke-exposed rats seemed physically destroyed, whereas the NanoMC-treated groups demonstrated superior preservation of islet architecture with fewer degenerative and necrotic cells. This suggests that NanoMC may help to protect the pancreas in diabetes situations exacerbated by cigarette smoke exposure. The findings of this study found that the strongest overall response was seen at 80 mg/kg BW NanoMC, indicating a dose-dependent therapeutic propensity.

Since the design of this study was focused on evaluating therapeutic effects under combined diabetes and cigarette smoke exposure rather than isolating the independent effect of cigarette smoke, there is an absence of a diabetic group without cigarette smoke exposure, and we acknowledged it as a study limitation. Although previous studies have reported that cigarette smoke and diabetes are associated

with oxidative stress, inflammation, insulin resistance, and beta-cell dysfunction (2,4-7,30); the present study did not measure malondialdehyde (MDA), 8-hydroxy-2'-deoxyguanosine (8-OHdG), superoxide dismutase (SOD), tumor necrosis factor (TNF)-alpha, or interleukin (IL)-1β), or other oxidative-inflammatory biomarkers.(21) The absence of these parameters is also an important limitation of this study. Future experiments should also incorporate plasma or urinary nicotine and/or carboxyhemoglobin to confirm smoke exposure, alongside oxidative-stress and inflammatory biomarkers, and should use prespecified blinded histomorphometry or immunohistochemical beta-cell markers.

Conclusion

In this animal model, NanoMC could improve glycemic and lipid-profile parameters in cigarette smoke-exposed diabetic rats, with the most favorable overall findings observed at 80 mg/kg BW. Semiquantitative analysis of pancreatic cell damage also suggested better preservation of pancreatic architecture, as indicated by lower islet of Langerhans damage scores and fewer degenerative or necrotic changes in treated groups. These findings support the potential of NanoMC that could be a phytochemical agent for diabetes-related metabolic and pancreatic tissue alterations in experimental models.

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Authors Contribution

YK, LY, RAI, MMD, YA, RS, SD, and RF were involved in the conception and design of the research. LY, RAI performed data acquisition. YK, MMD, RAI were contributed to the data analysis and interpretation of the results.YK, LY, RAI, MMD, YA, RS, SD, and RF were involved in manuscript preparation. YK, RAI, and RS designed the figures and/or tables. All authors participated in the critical revision of the manuscript and approved the final version.

Ethical Statement

This experimental animal study was approved by the Ethics Committee with Ethical Clearance Number 272/KEPK-Unisba/X/2024.

Conflict of Interest

The authors declare no conflicts of interest.

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